Final Report to the Midwest Forensics Resource Center

1. Determination of Heavy Metals in Whole Blood using Inductively Coupled Plasma-Mass Spectrometry

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3. Final Report: **April 1-June 30, 2008**

4. Project Description:

This project addresses needs in the area of forensic toxicology for the development of suitable methods for assessing inorganic toxicity, with a particular emphasis on heavy metals. This work proposes to employ the high sensitivity, wide dynamic range and multielement capability of inductively-coupled plasma mass spectrometry (ICP-MS) for the determination of metals and metalloids in whole blood.

A method for the quantitation of arsenic (As), cadmium (Cd), mercury (Hg), thallium (Tl) and lead (Pb) will be developed. The capability to add elements to those in this suite will also be pursued.

5. Project Objectives:

The objectives of the project are three-fold:
A) Develop a quantitative method for As, Cd, Hg, Tl and Pb in whole blood
B) Assess the use of microwave-assisted digestion of whole blood samples in addition to currently used dilution protocols.
C) Use the results of acquired data to develop an inorganic database for the forensic toxicology unit.

6. Results/Discussion

Parts A and B of the objectives were essentially completed. Due to serious instrument malfunctions in the last period of the grant, full validation statistics were not obtained and the database for the toxicology unit was not developed. This work will continue post-funding following instrument overhaul. Data is presented as obtained to date for the microwave and dilution methods.

Microwave Digestion vs. Direct Analysis (dilution)

Two separate methods for the determination of metals in blood have been developed. The first method employs microwave digestion of whole blood samples in HNO_3/H_2O_2, followed by matrix-matched standard additions analysis in the ICP-MS. The second

method involves direct analysis of whole blood following dilution with a diluent, also employing matrix-matched standard additions analysis.

The advantage of the microwave method is the overall reduction in dissolved solids afforded by the complete digestion of all biological material. A lower dilution factor (1/10) can be used compared to the dilution method, resulting in greater sensitivity. Problems with nebulizer clogging and the build-up of deposits on the torch and cones are greatly reduced. This leads to more maintenance-free operation of the ICP-MS and is accompanied by a reduction in the instability of the plasma and the resultant signal.

The disadvantage of the microwave method is the significant amount of time required for sample preparation and digestion. Extensive pipetting is involved in preparing for digestion, and a large amount of time is involved in readying the digestion bombs. In addition, the digestion apparatus used in this study has space for just ten samples. This requires two digestion cycles for sufficient space to prepare the standard curve, QC standards, SRM, and ten actual samples. The amount of cooling time following the digestion also adds to the length of the process.

The advantage of the dilution method is the relatively rapid and easy preparation of samples. However, due to dissolved solids, a greater dilution factor (1/15) compared to the microwave method is required. The biological material in the samples tends to clog the nebulizer more easily, and the associated increase in deposits on the cones and torch requires greater between-run maintenance of the instrument.

Linearity

Both methods were linear ($R^2 = 0.999$) over the ranges calibrated. Arsenic was calibrated from 10-250 ug/L, Cd and Tl were calibrated from 1-10 ug/L; Hg was calibrated from 1-100 ug/L; and Pb was calibrated from 10-250 ug/L. The lower limit of calibration for Hg was changed to 3 ug/L later in the work.

These calibration ranges were designed to detect an acute, massive exposure of a subject to these metal toxins, as opposed to monitoring lower levels of chronic exposure, i.e. biomonitoring. These ranges were selected based on literature values from a biomonitoring study of 100 normal healthy subjects (1) for As, Cd, Tl and Pb. Mercury ranges were selected based on methods from the Centers for Disease Control. These calibration ranges exceed those of the biomonitoring studies by up to several orders of magnitude. No deviations from linearity were observed even at the highest calibrators.

Metal	Reference Range (ug/L)	Upper Calibrator (ug/L)
As	2.6-17.8	250
Cd	0.15-2.04	10
Hg	3-10	100
Tl	0.011-0.035	10
Pb	11.4-62.8	250

1) Goulle J-P, Mahieu L, Castermant J, et al; Metal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair: reference values; For. Sci. Int. 153 (2005) 39-44.

Limit of Quantitation

The limit of quantitation (LOQ) is estimated as three times the standard deviation of the low QC bench standard for each method. With the exception of arsenic, improvements in LOQ are seen for the microwave method compared to the dilution method.

LOQ (ug/L)	Microwave Method	Dilution Method
As	8.2	7.8
Cd	0.7	1.0
Hg	2.7	2.9
Tl	0.2	0.9
Pb	6.1	14.7

Detection Limits

The detection limits for each element are calculated based on a series of blanks. The detection limit is calculated as the mean blank signal plus three times the background. In some cases (*) the detection limit exceeds the LOQ as estimated by the deviation in the low bench standard. The general trend is for lower detection limits in the microwave method compared to the dilution method. This is somewhat expected given fewer matrix issues with the microwave digestion method.

DL (ug/L)	Microwave Method	Dilution Method
As	1.3	5.2
Cd	0.3	1.9*
Hg	1.3	4.6*
Tl	0.6*	0.4
Pb	2.1	4.4

Accuracy and Precision

Tables 1A-E summarize the data obtained for both methods. The 95% confidence interval is given for each method for each standard. The number of triplicate analyses is given in the parenthesis.

Analysis of the medium and high bench QC standards exhibit very good accuracy for both the dilution and microwave digestion methods; however, the microwave method consistently provides statistically significantly ($p < 0.05$) better precision (RSD from 2 to 10%) than the dilution method (RSD from 8 to 45%).

The bulk of the accuracy data for the microwave method falls within 15% of the true value or within 20% near the LOQ. A notable exception is the SRM designated as QMEQAS07B-03, which is uniformly in error, suggesting a potential problem with that SRM. There are some problems with mercury, but these are related to the discussion later in the report concerning the stability of mercury standard solutions. Accuracy values for the dilution method also fare well. The same problems with QMEQAS07B-03 and mercury are present. In general, further analysis of this SRM would contribute to better understanding of this outlier.

There are several notes for Tables 1A-E. The large RSD for the low bench QC Hg standard (@) likely results from a matrix effect impacting the detection limit for the dilution method. The 95 % confidence interval of the microwave digestion method (#) does not encompass the known low bench As standard value. The majority of experimental SRM (*) 95 % intervals overlap with the 95% confidence intervals of the SRM without containing the mean value.

TABLES 1A-E. The 95% Confidence Intervals of the Microwave and Dilution Methods

Arsenic-corrected	Accepted Value (µg/L)	Microwave Method (µg/L)	Dilution Method (µg/L)
Low bench QC	50 ± N/A	# 44.8 ± 2.1 (9)	47 ± 4 (4)
Medium Bench QC	100 ± N/A	99.3 ± 2.3 (9)	104 ± 13 (4)
High Bench QC	200 ± N/A	198 ± 5 (9)	220 ± 30 (4)
QMEQASO7B-06	13.5 ± 2.6	*16.1 ± 1.5 (5)	18 ± 5 (4)
QMEQASO6B-08	8 ± 3	9.2 ±1.0 (2)	*13.5 ± 0.9 (1)
QMEQASO7B-03	8.2 ± 3.0	*4.8 ±1.0 (2)	8.7 ± 1.0 (1)

^{111}Cd	Accepted Value (µg/L)	Microwave Method (µg/L)	Dilution Method (µg/L)
Low bench QC	2.0 ± N/A	# 1.77 ± 0.17 (9)	1.6 ± 0.5 (4)
Medium Bench QC	5.0 ± N/A	4.8 ± 0.2 (9)	4.8 ± 0.6 (4)
High Bench QC	8.0 ± N/A	7.7 ± 0.4 (9)	7.9 ± 0.6 (4)
QMEQASO7B-06	14.0 ± 1.1	*12.8 ± 0.8 (5)	12.8 ± 2.5 (4)
QMEQASO6B-08	2.0 ± 0.3	2.6 ± 0.7 (2)	*1.60 ± 0.17 (1)
QMEQASO7B-03	1.31 ± 0.28	*0.9 ± 0.3 (2)	*0.90 ± 0.25 (1)

^{202}Hg	Accepted Value (µg/L)	Microwave Method (µg/L)	Dilution Method (µg/L)
Low bench QC	2.0 ± N/A	1.6 ± 0.7 (9)	@ 0.9 ± 2.4 (4)
Medium Bench QC	10.0 ± N/A	11.0 ± 1.0 (9)	10.5 ± 1.1 (4)
High Bench QC	25.0 ± N/A	20 ± 6 (9)	22 ± 10 (4)
QMEQASO7B-06	25 ± 3	*28.8 ± 2.4 (5)	23 ± 8 (4)
QMEQASO6B-08	11.0 ± 1.0	*9.4 ± 0.8 (2)	11.20 ± 0.17 (1)
QMEQASO7B-03	64 ± 4	*54.5 ± 0.7 (2)	*66.6 ± 1.0 (1)

^{205}Tl	Accepted Value (µg/L)	Microwave Method (µg/L)	Dilution Method (µg/L)
Low bench QC	2.0 ± N/A	2.09 ± 0.06 (9)	1.8 ± 0.5 (4)
Medium Bench QC	4.0 ± N/A	4.17 ± 0.18 (9)	4.0 ± 0.7 (4)
High Bench QC	6.0 ± N/A	6.24 ± 0.18 (9)	6.1 ± 0.7 (4)
QMEQASO7B-06	2.91 ± 0.17	2.92 ± 0.17 (5)	*2.4 ± 1.3 (4)
QMEQASO6B-08	1.90 ± 0.18	*1.72 ± 0.02 (2)	*1.50 ± 0.07 (1)
QMEQASO7B-03	1.92 ± 0.20	*1.76 ± 0.13 (2)	*1.60 ± 0.07 (1)

^{208}Pb	Accepted Value (µg/L)	Microwave Method (µg/L)	Dilution Method (µg/L)
Low bench QC	25 ± N/A	23.2 ± 2.0 (9)	23 ± 8 (4)
Medium Bench QC	100 ± N/A	104 ± 3 (9)	106 ± 12 (4)
High Bench QC	200 ± N/A	206 ± 6 (9)	213 ± 21 (4)
QMEQASO7B-06	400 ± 30	376 ± 23 (5)	360 ± 70 (4)
QMEQASO6B-08	100 ± 5	*93.1 ± 1.6 (2)	*88.40 ± 0.10 (1)
QMEQASO7B-03	100 ± 9	*73 ± 6 (2)	*89.6 ± 0.9 (1)

Water and Beverage Analysis: Based on a request from a submitting agency, a protocol for the analysis of water or clear beverages for the presence of As, Se, Cd, Tl and Pb was developed using the basic outline of the blood methods. This request resulted in a case report. This technique will be considered as a routine offering of the Trace Unit.

7. Dissemination

This work was presented as a poster session at the Midwest Association of Forensic Scientists 2007 annual meeting in Traverse City, MI in September 2007. This work was also presented at the annual American Academy of Forensic Science meeting in Washington DC in February 2008 as part of the Young Forensic Scientist forum. Further dissemination plans (co-PI funded) include presentations of the completed project at the Midwest Association of Forensic Scientists 2008 meeting in Des Moines, IA and the

Pittsburgh Conference in Chicago 2009 and/or the AAFS annual meeting in Denver, CO.

8A. Brief Quarter Expenses Summary

A separate report will be filed by the financial department of the Wisconsin Department of Justice.

8B. Discussion of Problems that Have Arisen:

A number of problems arose in the last period of the grant. Problems were encountered with mercury, with cadmium, with the base blood and with the ICP-MS itself.

a) Apparent problems originally attributed to the well-known mercury "memory" effect continued. The diluent and wash solutions were made to 200 ug/L with Au, a published remedy for this effect. However, problems with the standard curve and the values of QC samples and SRMs indicated that the problem might exist with the calibration standards themselves. It appears, although this has not been fully tested, that the mercury in the calibration intermediate stock solutions is much less stable than those of the other analytes. While As, Cd, Tl and Pb were stable for periods up to nine months, the mercury solutions appear to be stable for only 2-3 months. Improved results were obtained with more freshly made calibrators.

b) The use of the more abundant cadmium isotopes, Cd-112 and Cd-114, require a correction for tin, which presents an isobaric interference. However, high background tin levels existed in the laboratory blank during the time of analysis. This has been tentatively attributed to the construction wok taking place in the building during this period, where, perhaps, a high amount of tin could have been present from soldering. The remedy was to use the slightly less abundant isotopes of cadmium, namely, Cd-111 and Cd-113, which have no isobaric interference from tin. This correction was successful.

c) It was discovered that the porcine base blood, as prepared in the toxicology unit, is routinely diluted by a factor of 1/3 with normal saline. In early trails of the dilution method, base blood and casework samples were diluted to the same extent without regard to the initial dilution of the base blood. This resulted in a higher and unacceptable level of dissolved solids in the ICP-MS nebulizer. The remedy was to simply dilute the porcine blood 1/10 and the casework human blood 1/15, matching the samples with respect to dissolved solids level and total dilution.

d) The most serious problem encountered, one which prevented completion of the project, was the increasing instability of the plasma in the ICP-MS itself. Analysis RSDs increased in March; the recommended maintenance procedures were implemented. In April, the plasma became vey unstable to the point where the plasma would extinguish during tuning procedures or during unattended autosampler runs. A visit from the Perkin-Elmer service engineer did not resolve the problem, although either the RF generator or the mass flow controller for the argon supply was implicated.

9. Future of the Project

The future of the project lies primarily in correcting the plasma instability of the ICP-MS so that work can proceed. The funds for this will probably be available in the next fiscal year. Work will continue under the aegis of the toxicology unit on an unfunded basis with the goal of completing the task of adding the blood metal analysis methods to the toxicology unit manual.